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RESEARCH ARTICLE

# Motor skill learning depends on protein synthesis in the dorsal striatum after training

**Tobias Wächter · Sebastian Röhrich · Anita Frank · Katiuska Molina-Luna · Ana Pekanovic · Benjamin Hertler · Maximilan Schubring-Giese · Andreas R. Luft**

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**Abstract** Functional imaging studies in humans and electrophysiological data in animals suggest that cortico-striatal circuits undergo plastic modifications during motor skill learning. In motor cortex and hippocampus circuit plasticity can be prevented by protein synthesis inhibition (PSI) which can interfere with certain forms learning. Here, the hypothesis was tested that inducing PSI in the dorsal striatum by bilateral intrastriatal injection of anisomycin (ANI) in rats interferes with learning a precision forelimb reaching task. Injecting ANI shortly after training on days 1 and 2 during 4 days of daily practice ($n = 14$) led to a significant impairment of motor skill learning as compared with vehicle-injected controls ($n = 15$, $P = 0.033$). ANI did not affect the animals' motivation as measured by intertrial latencies. Also, ANI did not affect reaching performance once learning was completed and performance reached a plateau. These findings demonstrate that PSI in the dorsal striatum after training impairs the acquisition of a novel motor skill. The results support the notion that plasticity in basal ganglia circuits, mediated by protein synthesis, contributes to motor skill learning.

T. Wächter · S. Röhrich · A. Frank · K. Molina-Luna · A. Pekanovic · B. Hertler · M. Schubring-Giese · A. R. Luft (✉)
Department of Neurology, Clinical Neurorehabilitation, Rehabilitation Institute and Technology Center Zurich (RITZ), University of Zurich, Frauenklinikstr. 26, 8091 Zurich, Switzerland
e-mail: andreas.luft@usz.ch

T. Wächter
Department of Neurodegeneration, Center of Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Tübingen, Germany
e-mail: tobias.waechter@uni-tuebingen.de

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## Introduction

Several brain areas interact to enable the acquisition of motor skills. The sensorimotor cortex interacts with the basal ganglia (Pisani et al. 2005; Costa 2007), the cerebellum and other sensory as well as associative cortical areas (Hikosaka et al. 2002). Imaging studies in humans demonstrate activation of the striatum during skill learning (Seitz et al. 1990; Lehericy et al. 2005; Wachter et al. 2009). These studies also suggest that striatal circuits undergo plastic modifications as neuronal activation shifts from the associative, rostrodorsal region to the sensorimotor, caudoventral region of the putamen (Lehericy et al. 2005) in association with learning a new finger tapping sequence. Evidence for this shift of activation was also found in non-human primates using single cell recordings (Miyachi et al. 2002). Striatal activation changes corresponded to the individual learning rate in primates (Brasted and Wise 2004) and mice (Costa et al. 2004) suggesting a link between plastic modification in this area and skill learning.

Certain forms of circuit plasticity depend on neuronal protein synthesis (Davis and Squire 1984; Steward and Schuman 2001). Inhibition of protein synthesis in the entire body or within certain brain areas leads to learning impairments. Infusion of the protein synthesis inhibitor anisomycin (ANI) into the amygdala interferes with memory consolidation in classical conditioning paradigms (Nader et al. 2000). Instrumental conditioning is impaired by injections into the nucleus accumbens (Hernandez et al. 2002). In the latter study instrumental learning deteriorated only

after ANI injections into the ventral (associative) but not the dorsal (motor) striatum.

Because of the close connectivity between the dorsal striatum and motor cortical areas, we hypothesized that ANI injected into dorsal striatum would impair skill acquisition similar to its effect in motor cortex that we have reported previously using comparable experimental protocols (Luft et al. 2004).

## Materials and methods

### Animals and protocols

All experiments were performed in adult male Long-Evans rats (8–10 weeks, 250–350 g body weight) raised in our animal facility. Animals were housed individually in a 12/12-h light/dark cycle (light on: 3 a.m., off: 3 p.m.). All procedures were approved by the Animal Care Committee of the State of Baden-Württemberg.

Twenty-nine rats were randomly assigned to receive either injections of ANI ($n = 14$) or saline (control, $n = 15$) into the dorsal striatum immediately after training on days 1 and 2, i.e., during the time of greatest skill improvement (=learning). An additional nine animals were injected with ANI into dorsal striatum after training on days 10 and 11 in the plateau phase when no further learning occurred. The latter experiment was conducted to test whether ANI in dorsal striatum affects motor performance thereby artificially influencing the learning curve.

### Motor skill learning

Training sessions were performed at the beginning of the dark phase. Animals were food restricted for 24 h before the first pre-training session (see below). During training animals were kept slightly over their initial weight by providing 40–50 mg/kg of standard lab diet after each training session. Water was given ad libitum.

Motor skill training was performed as previously described (Buitrago et al. 2004). Briefly, 5 days of pre-training were followed by a 6 to 15-day training period. During pre-training rats had to learn to open a motorized door covering a window in the cage front wall that gave access to food pellets. The door was opened by nose poking a sensor in the rear wall. Food pellets were retrieved with the tongue. Training was then initiated by moving the pellet-holding pedestal 1.5 cm away from the window. In this position pellets could only be retrieved with the forelimb. After determining forelimb preference, the pedestal was shifted to one side of the window to allow reaching only with the preferred limb. Each reaching trial was scored as "successful" (reach, grasp and retrieve) or "unsuccessful" (pellet pushed off pedestal or dropped during retraction). The index of performance was the ratio between the number of successful trials and the total number of trials per session. The latency between pellet removal and subsequent door opening was used as an index of motivation (Buitrago et al. 2004). Daily training sessions consisted of 100 door openings ($117.2 \pm 28.9$ reaching movements, mean $\pm$ SD, automatically sensed by a sensor between cage wall and pedestal) and lasted $24.4 \pm 12.7$ min (mean $\pm$ SD).

### Surgery

After pre-training, animals underwent stereotaxic placement of guide cannulas (Unimed, Lausanne, Switzerland; diameter 400 μm) into dorsal striatum bilaterally (coordinates relative to bregma, 3 mm lateral, 0 mm anterior–posterior, 5 mm deep). The coordinates for implantation were selected on the basis of a rat brain atlas (Paxinos and Watson 1998). For implantation animals were anaesthetized with ketamine (i.p., 10%, i.p., 70–100 mg/kg body weight) and xylazine (2%, i.p., 5–10 mg/kg body weight). Additional ketamine doses were administered if necessary. Body temperature was measured rectally and maintained using a water heating pad at $37 \pm 0.5$°C. Cannulas were slowly inserted through two burr holes (1.5 mm diameter) using manual micropositioners. The cannulas were fixed in place using bone cement (FlowLine, Heraus Kulzer, Dormagen, Germany). Buprenorphin (0.01 mg/kg, i.p.) was given after surgery for pain relief and all animals were recovered for 3 days before motor skill training was started.

At the end of the training animals were euthanized and correct position of the cannulas was confirmed in all animals by histology (Nissl stain; Fig. 1).

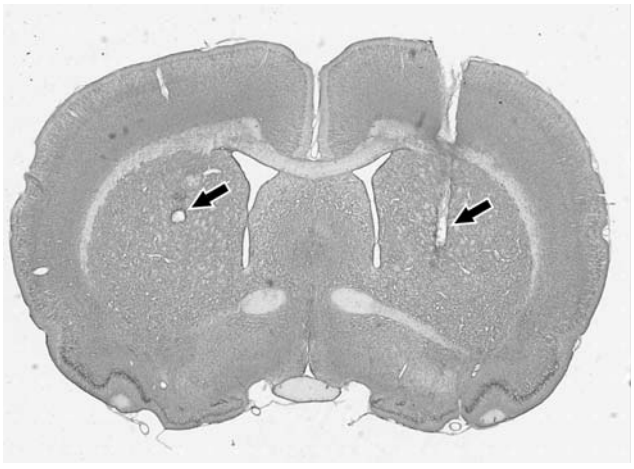

**Fig. 1** Example of histological brain section (Nissl stain) to confirm positioning of cannulas in the dorsal part of the striatum bilaterally. *Arrows* point to position of cannulas

### Intrastriatal injections

Immediately after training on days 1 and 2, rats were briefly anaesthetized with a combination of fentanyl (0.005 mg/kg), medetomidin (0.15 mg/kg), and midazolam (2.0 mg/kg). Under sedation either 0.5 µl ANI dissolved in 1 µl phosphate buffer solution ($n = 14$) or saline ($n = 15$) was injected using a microinjection pump (Nano-injector, Stoelting Co., Wood Dale, IL, USA) and a micro-syringe (5 µl, Hamilton, Martinsried, Germany) connected to a thin needle (35 gauge, Hamilton). The injection speed was 0.1 µl/min. One additional minute with the injection needle in place was allowed for spread and diffusion of the drugs. In order to terminate the anesthesia after successful microinjection, animals received an antidote (s.c.) consisting of a combination of atipamezole (0.75 mg/kg), flumazenil (0.2 mg/kg), and naloxone (0.12 mg/kg). The time elapsed between the last reach and successful injection was carefully monitored and shorter than 20 min for all cases.

### Statistical analysis

Data analysis was performed using SPSS version 16 (SPSS Inc., Chicago IL, USA). Reaching performance was quantified as the percentage of trials with successful retrievals per session (=100 trials). General linear repeated measures models were used to test for effects of *training day* on *reaching performance* including *group, baseline performance*, and the interaction of group × time (session) as independent variables. Only the initial four sessions were used to test for the interaction of group × time as ANI was injected on days 1 and 2 and our previous data demonstrate, that the protein synthesis normalizes approximately 48 h after the last injection. Furthermore learning in the reaching task typically occurs within those initial four sessions. Thereafter animals reach a plateau (Buitrago et al. 2004). We did not expect ANI to affect the plateau, only the steepness of the initial learning curve. Whether data met the sphericity condition was tested using Mauchly's criterion and if not met, Geisser and Greenhouse (1959) correction was applied. To compare the effects of protein synthesis inhibition (PSI) in dorsal striatum to those observed after PSI in motor cortex in our prior study, partial eta square values were computed for the group × time interaction effect in the general linear model (including the initial 4 sessions). Two-tailed probability ≥5% was considered significant.

## Results

Inhibiting protein synthesis in dorsal striatum after training on days 1 and 2 impaired motor skill acquisition as compared to controls (Fig. 2a). Considering the initial four sessions, the interaction effect of group × time was significant, $F(3,78) = 3.06$, $P = 0.033$ (observed power 0.70). Comparing all 10 sessions resulted in a statistical trend without reaching the 5% significance level, $F(9,234) = 15.9$, $P = 0.075$ (power 0.79).

Protein synthesis inhibition after learning, when performance had reached a plateau, did not result in impaired reaching performance (Fig. 2b, difference between the average performance on days 9 and 10, before injection, and day 11, after first injection: $P = 0.58$, day 12, after second

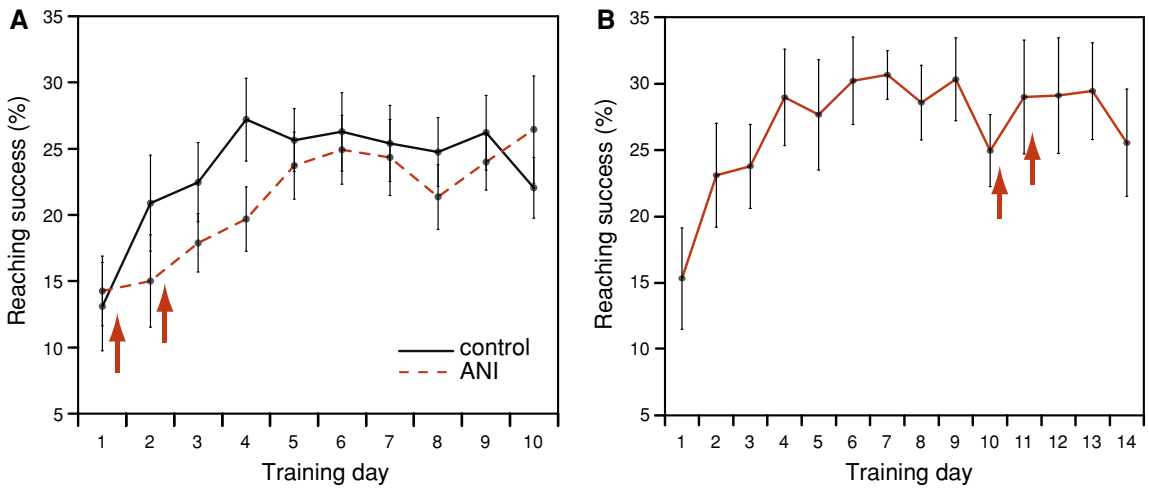


**Fig. 2** Protein synthesis inhibition in bilateral dorsal striatum impairs the acquisition of a forelimb reaching skill in rat. **a** Animals injected with anisomycin into dorsal striatum after training on days 1 and 2 show slowed motor skill acquisition. **b** PSI in the dorsal striatum after training on days 10 and 11, however, does not lead to a deterioration of reaching performance, indicating that PSI in the dorsal striatum specifically impairs learning but not task execution. *Error bars* indicate standard error

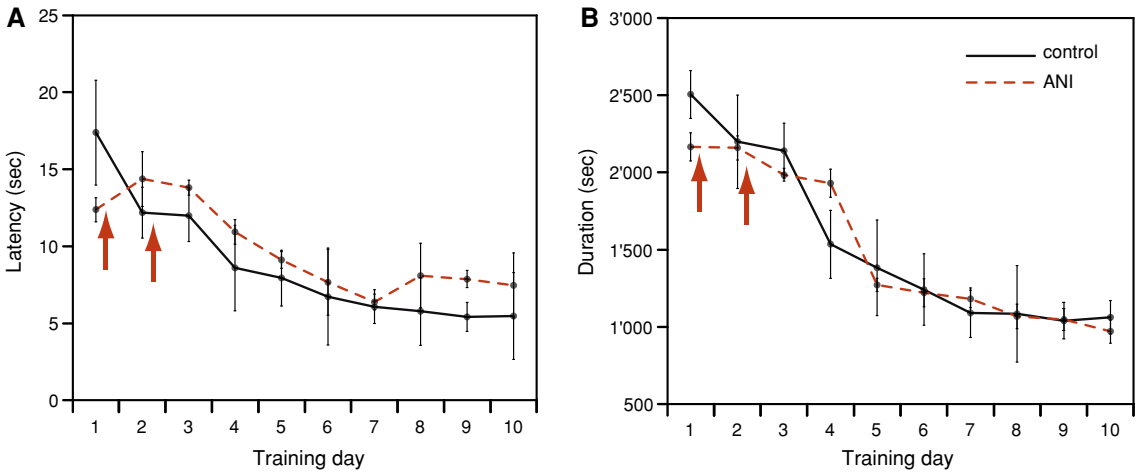


**Fig. 3** Effect of ANI on intertrial latencies and session duration. **a** Intertrial latencies and **b** session duration are not affected by ANI injections into bilateral dorsal striatum. *Error bars* indicate standard error

injection: $P = 0.50$, day 13: $P = 0.34$) indicating that protein synthesis in basal ganglia was required for skill acquisition but not mere motor performance.

In contrast to its effect on skill learning, PSI did not affect the latencies between reaching trials, i.e., the intervals between pellet removal—successful or unsuccessful—and subsequent door opening (Fig. 3a) nor did it affect the total duration of the training session (Fig. 3b). Repeated measures ANOVA revealed no effect of group on time, $P = 0.41$ (power 0.26), while in both groups the latencies decreased at a similar rate, $F(9,243) = 8.27$, $P < 0.001$ (power 0.99, effect of time). Similarly, for duration there was no effect of group on time, $P = 0.40$ (power 0.27), but an overall effect of time, $F(9,243) = 30.2$, $P < 0.001$ (power 1.0). These findings indicate that motivation of the animal and their general knowledge of the task (how to open the door, where to look for the pellet) was unaffected by PSI in basal ganglia.

Comparing the learning impairment induced by PSI in striatum with that produced by PSI in primary motor cortex using analogous methods (Luft et al. 2004) showed that the effect of PSI was more pronounced in cortex than in striatum, effect size for cortex $\eta_p^2$ =0. 131 [effect of group × time, initial 4 sessions: $F(3,27) = 3.76$, $P = 0.022$], for striatum $\eta_p^2$ =0.105 [effect of group × time, initial 4 sessions: $F(3,78) = 3.06$, $P = 0.033$].

## Discussion

We found that inhibition of protein synthesis in the dorsal striatum, outside of a training session, impaired the acquisition of a novel motor skills, which then recovered after 4 days. Intact protein synthesis in the striatum is therefore required for motor skill learning.

These findings corroborate to data from humans and animals suggesting that striatal plasticity contributes to skill learning. Imaging studies show changes in the activation of the dorsal striatum (Lehericy et al. 2005) and electrophysiological experiments in animals show evidence for reorganization of corticostriatal circuits during early stages of motor skill learning (Brasted and Wise 2004; Costa et al. 2004; Yin et al. 2009).

Volume and concentration of ANI injected into striatum was identical to our previous experiment with ANI injections in the cortex and cerebellum (Luft et al. 2004) as well as to the study by Hernandez et al. (2002) that reported injections into ventral and dorsal striatum. The spread of the substance is mainly driven by the injection pressure. Using India ink this spread was previously simulated in cortex and cerebellum to be approximately 2 mm in diameter. Assuming a similar spread for the basal ganglia, we believe that the tissue most directly affected by the injections was the dorsal striatum. Although subsequent diffusion extends the ANI effect to a larger tissue volume, the tissue block affected by relevant PSI in the cortex is smaller than 4 × 5 $mm^2$ (Luft et al. 2004). As the diameter of the dorsal striatum extends approximately 5 mm, we assume that ANI does not affect tissue outside the dorsal striatum. Hence given that the injections were delivered to the middle of the dorsal striatum, one can expect the relevant ANI effect to be restricted to the dorsal striatum.

Our data seems to contrast with the results of Hernandez et al. (2002) who found impairments in instrumental conditioning after ANI injections into the ventral striatum (core of the nucleus accumbens), but not after injections into the dorsal striatum. However, the differences are likely explained by the different forms of learning investigated in the two studies. Hernandez et al. (2002) used an instrumental conditioning paradigm while we studied the learning of

a novel precision motor skill. Our paradigm does include certain components of instrumental conditioning ("nose-poke the sensor to open the door to gain access to the pellet"), but these are not quantified using reaching success rates. We argued previously that instrumental conditioning components are measured by intertrial latencies (Buitrago et al. 2004). Here, intertrial latencies were unaffected by ANI injections into dorsal striatum. Therefore, protein synthesis in the dorsal striatum may be specifically required for motor skill learning but not for instrumental conditioning. This conclusion implies that the two forms of learning have different neuronal substrates: While instrumental conditioning depends on the formation of associations within limbic structures, motor skill learning may require modification in motor circuits formed between cortex and dorsal striatum.

It is interesting to note that PSI in dorsal striatum impaired learning between days 1 and 2 here, despite being induced after training on day 1 (reaching improvement between days 2 and 3 was also lower, but it cannot be discerned whether this due to injection one, 23 h before session 2, or injection two, immediately after session 2). A similar finding was obtained in our previous study of ANI injections into primary motor cortex (Luft et al. 2004). It is known that the formation of a skill memory happens not only during training but also during the rest (Shadmehr and Holcomb 1997) and the sleep phases thereafter (Walker et al. 2003). Interfering with these processes that may reflect consolidation between training sessions, may have led to impaired acquisition of the skill here.

The effect of PSI in the dorsal striatum on motor skill learning is smaller than effect induced by inhibition in motor cortex (Luft et al. 2004). This interpretation is limited because the experiments were not performed within the same study. Nevertheless, the differences may be the consequence of different plastic processes in striatal versus cortical networks. Also, plasticity requiring protein synthesis may occur in striatum at a different time during learning than the early phase targeted by ANI here.

While PSI in motor cortex produced a learning deficit that did not recover within eight training days, striatum-injected animals recovered control performance already after 4 days. As demonstrated for motor cortex, protein synthesis is restored 48 h after the second ANI injection (Luft et al. 2004). Assuming that protein synthesis is similarly restored in striatum, this time frame coincides well with the recovery of motor learning. It is unclear at this point why recovery from PSI in the striatum occurs sooner than recovery from PSI in motor cortex.

In conclusion, we found evidence that protein synthesis occurring in dorsal striatum during the rest phase between daily training sessions is necessary for normal motor skill learning. It remains to be elucidated which proteins are expressed and what role they play in the neuronal plasticity that underlies motor learning.

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